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Microbial communities of a native perennial bunchgrass do not respond consistently across a gradient of land-use intensification

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Abstract

To test if native perennial bunchgrasses cultivate the same microbial community composition across a gradient in land-use intensification, soils were sampled in fall, winter and spring in areas under bunchgrasses ('plant') and in bare soils ('removal') in which plots were cleared of living plants adjacent to native perennial bunchgrasses (*Nassella pulchra*). The gradient in land-use intensification was represented by a relict perennial grassland, a restored perennial grassland, and a perennial grass agriculture site on the same soil type. An exotic annual grassland site was also included because perennial bunchgrasses often exist within a matrix of annual grasses in California. Differences in soil resource pools between 'plant' and 'removal' soils were observed mainly in the relict perennial grassland and perennial grass agriculture site. Seasonal responses occurred in all sites. Microbial biomass carbon (C) and dissolved organic C were greater under perennial bunchgrasses in the relict perennial grassland and perennial grass agriculture site when comparing treatment means of 'plant' vs. 'removal' soil. In general, soil moisture, microbial respiration, and nitrate decreased from fall to spring in 'plant' and 'removal' soils, while soil ammonium and net mineralizable nitrogen (N) increased only in 'plant' soils. A canonical correspondence analysis (CCA) of phospholipid fatty acid (PLFA) profiles from all sites showed that land-use history limits the similarity of microbial community composition as do soil C and N dynamics among sites. When PLFA profiles from individual sites were analyzed by CCA, different microbial PLFA markers were associated with *N. pulchra* in each site, indicating that the same plant species does not retain a unique microbial fingerprint across the gradient of land-use intensification.

Keywords: *Nassella pulchra*; Annual grassland; Microbial community composition; Soil respiration; Nitrogen mineralization; Land use; PLFA; Agriculture

1. Introduction

Grasslands are unique ecosystems because they can have large belowground inputs of plant-derived labile carbon (C) in relation to the size of the soil organic matter (SOM) pool (Zak et al., 2000). Relict perennial grasslands in Mediterranean environments are usually composed of bunchgrasses surrounded by either bare soil or ephemeral annual plants (Jackson, 1985; Bouchet et al., 1999; Le Houerou, 2000). This bunchgrass arrangement leads to spatial heterogeneity, creating patches of higher soil C availability under bunchgrasses (Hook et al., 1991, 1994). Due to the spatial variation in soil C availability, relict perennial bunchgrasses may support unique plant and microbial communities that perform specific functions compared to interstitial zones without bunchgrasses. If so, the assemblages of microorganisms associated with a given species of perennial bunchgrass may be consistent from site to site. However, our previous evidence from relict bunchgrass sites in the Central Coast region of California indicates that the perennial bunchgrass, *Nassella pulchra*, has very different effects on microbial

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communities at different sites on the same soil type (Steenwerth et al., 2003). With respect to the many changes in the soil environment that occur after land-use conversion, should we expect to achieve the same microbial community composition and function in a restored site as in a relict, undisturbed grassland with *N. pulchra*? To examine this question, we investigated if *N. pulchra* influences microbial communities, activities and soil C and N pools similarly across a gradient of land-use intensification on the same soil type.

The effects that perennial bunchgrasses have on soil microbial community composition and diversity can be assessed by phospholipid ester-linked fatty acids (PLFA), which are quickly degraded in the soil environment and thus represent the living soil microbial community. The diverse set of PLFA from each soil sample can be analyzed by multivariate statistics to generate fingerprints of the microbial community (ter Braak, 1987). Microbial community fingerprints vary with the number, type and amount of detected PLFA in the soil.

Some recent evidence suggests that distinctive microbial populations can be present in the rhizosphere of specific plant taxa, but it is difficult to identify the factors responsible for these associations (Kowalchuk et al., 2002; Callaway et al., 2004; Batten et al., 2006). Plant species can differ in the production of labile C as root exudates, dead root cells, and fine root turnover (Shamoot et al., 1968; Robinson and Scrimegour, 1995; Marschner, 1998), but environmental factors and plant phenology can also affect these products (Fu et al., 2002).

Perennial bunchgrasses may change the microbial community, activity and nutrient pools compared to neighboring interstitial zones; yet, consistent changes across land-use types are unlikely due to the myriad of abiotic and biotic factors that occur in the plant–soil environment due to land-use history, even on the same soil type. To examine this hypothesis, we removed annual grasses and forbs from interstitial zones surrounding existing bunchgrass individuals of *N. pulchra* at three sites: a relict perennial grassland, a restored perennial grassland and an agricultural field of row-cropped perennial bunchgrasses. Our objectives were to determine if: (1) specific microbial PLFA were associated with *N. pulchra* compared to interstitial zones without plants, (2) soil resource pools and PLFA associated with *N. pulchra* differed seasonally, and (3) land use influenced the soil biodiversity associated with native bunchgrasses.

2. Materials and methods

2.1. Site description and climate

Locations of sites ranged from the Salinas Valley at 20 m elevation to the upper Carmel Valley at 556 m elevation (Monterey County, CA) (Steenwerth et al., 2003). The sites were located along a transect of approximately 30 km, moving eastward from Rana Creek Ranch near the town of Carmel Valley to the hills east of Gonzales. The restored perennial grassland ('Restored Grassland'), the perennial grass agriculture site ('Agricultural Grassland') and the relict perennial grassland ('Relict Grassland') were in Carmel Valley. The annual grassland ('Annual Grassland') was in Salinas Valley. The soils at all sites were derived from granitic and schistose alluvium. 'Relict Grassland' was on a transition zone between Gorgonio sandy loam soil (Sandy, mixed, thermic Fluventic Haploxeroll) and Santa-Lucia/Reliz variant (Clayey-skeletal, mixed, thermic Pachic Ultic Haploxeroll). 'Restored Grassland' was on Sheridan coarse sandy loam (Coarse-loamy, mixed, thermic Pachic Haploxerolls; Cook, 1978). 'Agricultural Grassland' was on Gorgonio sandy loam soil, while 'Annual Grassland' was on Chualar sandy loam soil (Fine-loamy, mixed, thermic Fluventic Haploxeroll). These soil series are classified on the basis of the whole pedon, and although they have different subsoil characteristics, their surface horizons where soil samples were collected are morphologically similar (Steenwerth et al., 2003).

The climate is Mediterranean, and precipitation typically occurs during the late fall through early spring followed by a summer drought. Precipitation during the rainy season from fall 2000 to spring 2001 (i.e., October 2000–March 2001, the period of study) was ca. 400 mm in Salinas Valley and Carmel Valley. The mean annual precipitation for the region is 520 mm (Cook, 1978). The mean daily temperature maxima range between 19.4 and 28.9 °C in the fall (September to November), 15.6 and 16.4 °C in the winter (December to February), and 16.3 and 22.2 °C in the spring (March to May).

The degree of soil disturbance differed between the four land-use types. 'Relict Grassland' supported purple needlegrass (*N. pulchra* (A. hitchc.) Barkworth), native annual and perennial forbs, and non-native annual grasses, and had never been tilled (see Table 1 for species list). At 'Agricultural Grassland', *N. pulchra* was grown for seed production and irrigated intermittently during March–April. 'Agricultural Grassland' was planted with *N. pulchra* for 5 years prior to the study, and several decades before that was cultivated for grain crops. *N. pulchra* was planted in rows along a ca. 75 cm wide bed in the field. Lastly, 'Restored Grassland' was cultivated by early settlers in Carmel Valley (mid-1800s to 1937). It then was abandoned and became an old-field annual grassland from approximately 1925–1995 (Stromberg and Griffin, 1996). Starting in November 1995, the soil was tilled and remained fallow for 2 years. It was tilled several times per year during the wet season and glyphosate (Roundup™) was applied as necessary to reduce the seedbank of annual grass species. In 1997, perennial bunchgrass (*N. pulchra* and *Elymus glaucus*) seed was drilled into the soil (Stromberg and Kephart, 1996). At the initiation of sampling in October 2000, perennial bunchgrasses had been established for 3 years. The 'Annual Grassland' was tilled once in the 1960s and then supported exotic European annual grasses that were grazed under a yearly rotation.

Table 1
Percent relative cover of plant species in the grasslands by the cover pin method ($n = 4$), April 2001

	Relict Grassland		Annual Grassland		Restored Grassland	
Plant species[a]	x	±SE	x	±SE	x	±SE
Bromus diandrus	4.2	1.5	29.6	5.0	1.2	0.3
Bromus hordeaceous	9.4	1.8	35.6	4.5	21.5	3.9
Erodium cicutarium	9.4	1.2	7.2	2.6	6.1	1.3
Vulpia myuros	1.1	nd	1.5	nd	24.5	1.7
Avena barbata	24.3	2.3	0.3	nd	nd	nd
Bromus madritensus v. rubens	2.3	0.0	7.0	nd	nd	nd
**Amsinckia menziesii*	10.9	1.5	nd	nd	3.0	0.7
**Clarkia purpurea*	0.9	nd	nd	nd	4.0	0.6
Hypochaeris glabra	5.0	0.0	nd	nd	2.1	1.3
**Nassella pulchra*	5.7	nd	nd	nd	5.5	2.7
Anagalis arvensis	nd	nd	0.1	nd	0.5	0.2
Eremocarpus setigerus	nd	nd	1.3	nd	0.3	nd
Medicago spp.	nd	nd	2.9	1.0	3.7	1.6
Poa annua	nd	nd	1.1	nd	0.7	0.5
**Dichelostemma capitatum*	3.5	2.4	nd	nd	nd	nd
**Eriogonum spp.*	8.1	4.3	nd	nd	nd	nd
Galium parisiense	8.3	1.8	nd	nd	nd	nd
**Lotus strigosus*	4.1	0.4	nd	nd	nd	nd
**Lotus wrangelianus*	5.2	4.4	nd	nd	nd	nd
Brassica nigra	nd	nd	4.7	0.8	nd	nd
Hordeum murinum	nd	nd	8.3	3.9	nd	nd
**Yabea microcarpa*	nd	nd	0.7	nd	nd	nd
Corethrogyne filagnifolia	nd	nd	nd	nd	1.0	0.5
**Elymus glaucus*	nd	nd	nd	nd	5.7	2.2
**Lupinus bicolor*	nd	nd	nd	nd	2.0	1.1
**Lupinus nanus*	nd	nd	nd	nd	15.9	3.1
**Micropus californicus*	nd	nd	nd	nd	1.9	1.0
**Plagiobothrys nothofulvus*	nd	nd	nd	nd	0.4	0.1
Stellaria media	nd	nd	nd	nd	0.1	nd
Unknown forb	nd	nd	nd	nd	0.1	nd
No. of native species	6		1		9	
Total species richness	15		13		20	

"nd" indicates that the species was absent. No measurements were made at the 'Agricultural Grassland' site due to weed control practices.
[a]Asterisks indicate that the species are native.

2.2. Sampling scheme and treatments

At each site in late September before the onset of fall rains, treatments with ('plant') and without ('removal') vegetation were established. The 'plant' plots contained the perennial bunchgrass, *N. pulchra*, or in the case of annual grassland, non-native annual grasses. Surface litter and dead annual grasses and forbs were gently removed by hand to minimize physical disturbance in the 'removal' plots. Seedlings were removed as they germinated during the study. By creating bare soil plots adjacent to the perennial bunchgrasses prior to the growth season, we tested the effects of the living plant and its associated rhizodeposits and litter ('plant') on soil resource pools and microbial communities vs. a soil environment with lower C inputs and no living plants or surface litter ('removal').

Each site contained four randomly chosen, paired 1.5–2 m^2 plots. The paired plots at each site were distributed within an area of ca. 600 m^2. Each pair consisted of a 'plant' and 'removal' plot. All 'removal' plots initially only contained annual plant species and were 1 m away from areas that contained annual and perennial grasses in all sites except 'Restored Grassland'. In 'Restored Grassland', due to the close spatial arrangement of bunchgrasses, removal samples were taken >30 cm from the bunchgrass. In 'Agricultural Grassland', plots were selected in areas where bunchgrasses were at least 1 m apart, and samples from 'removal' plots were collected on the bed. In the three sites supporting perennial bunchgrasses, soil samples were collected directly under *N. pulchra* in the root zone. All soil samples were cored to a depth of 12 cm from the surface in fall (October 20, 2000), winter (February 19, 2001) and spring (March 19, 2001). Excavation to 1 m depth and subsequent inspection of rooting depths in these sites showed that the majority of roots were concentrated in this zone (Potthoff et al., 2005; Steenwerth and Jackson, unpublished data). In addition, at each sampling in fall, winter, and spring, aboveground plant biomass was collected from three randomly selected 0.25 m^2 areas adjacent to the established 'plant' plots. In

early April 2001, plant community composition was determined by using a cover pin frame, which provides a measure of plant species frequency and relative cover (Kent and Coker, 1992). Pin frame measurements were taken at 10 randomly distributed positions along each of four transects per site (i.e., 40 pin frames per site). The site was divided into four quadrants and a transect (15 m in length) was placed randomly within the respective quadrant. Pin frames were treated as subsamples along each transect, and therefore were averaged to represent the relative cover of individual species for a given transect. Relative cover for each species at the site was then averaged by transect ($n = 4$).

2.3. Soil and plant analyses

Determination of microbial biomass C (MBC) was by fumigation extraction (Brookes et al., 1985; Vance et al., 1987), and dissolved organic carbon (DOC) was determined from unfumigated extracts. Organic C in the 0.5 M K_2SO_4 extracts was measured by analyzing diluted extracts (1:10) on a Phoenix 8000 UV enhanced-persulfate digestion TOC analyzer (Dohrmann [Tekmar-Dohrmann], Manson, OH) according to the method of Wu et al. (1990). Soil MBC was calculated from the relationship: biomass $C = E_C/k_{EC}$, where $E_C =$ [organic C extracted from fumigated soil]−[organic C extracted from non-fumigated soil] and $k_{EC} = 0.45$ (Wu et al., 1990; Joergensen, 1996; Potthoff et al., 2005). Soil respiration was measured by placing soil in sealed bottles and measuring the carbon dioxide (CO_2–C) concentration in the headspace after incubating at 25 °C for 60 min at field moisture. Respired CO_2–C was analyzed with a gas chromatograph (HP 5890A with TCD, Hewlett Packard, Palo Alto, CA). Inorganic N was extracted immediately with 2 M KCl in the field. Nitrate ($NO_3^- - N$) and ammonium ($NH_4^+ - N$) were analyzed with a Lachat Quick Chem II Flow Injection Analyzer (Zellwegger Analytical, Milwaukee, WI). Soil moisture, or gravimetric water content (GWC), was determined after drying soil at 105 °C for at least 48 h. Potential net N mineralization was performed by anaerobically incubating the soil for 7 days at 40 °C (Waring and Bremner, 1964), modified by blowing dinitrogen gas into the headspace for 30 s prior to closure of the 50 ml conical vial.

Soils were air-dried, and large roots (>1 mm) were removed with tweezers. Soils were sieved through a 2 mm mesh screen, and coarse fragments >2 mm were weighed. Sieved soils from each site were analyzed for pH by saturated paste (US Salinity Laboratory Staff, 1954), and for particle-size distribution by the method of Gee and Bauder (1986). Total soil C and N were determined by combustion using a Carlo Erba CN analyzer (Pella, 1990). All analyses were conducted by the DANR Lab (URL: danranlab.ucdavis.edu).

A subsample of field-moist soil was simultaneously obtained for PLFA analysis from each soil core (surface 0–12 cm). This soil was stored at −20 °C until extraction. Immediately before PLFA analysis, soil from each frozen sample was mixed, and all visible root fragments were removed with tweezers. Two replicates were analyzed per soil sample. A sample was also taken for gravimetric moisture by drying soil at 105 °C for 48 h. A complete description of the procedure for PLFA extraction and analysis is detailed in Bossio and Scow (1995). Fatty acid terminology utilizes "*A*:*B* ωC" where "*A*" indicates the total number of carbon atoms, "*B*" the number of unsaturations, and "ω" precedes "*C*", the number of carbon atoms between the closest unsaturation and the aliphatic end of the molecule. The suffixes "c" and "t" indicate cis and trans geometric isomers. The prefixes "i" and "a" refer to iso and anteiso methyl branching. Hydroxyl groups are indicated by "OH". Cyclopropyl groups are denoted by "cy". 10 Me refers to a methyl group on the 10th carbon from the carboxylic end of the fatty acid. Total extractable PLFA provided a second measure of microbial biomass (Zelles et al., 1995).

2.4. Statistical analysis

Statistical comparisons to test for differences between sites and effects of season and the 'plant' and 'removal' treatments on soil resource pools, moisture, and microbial activity were conducted by using a three-way general linear model (GLM) (SAS version 8.2, SAS Institute, Cary, NC, USA). The model tested for interdependence between replicates of 'plant'/'removal' treatments for a given site and over time. Results indicated that these could be treated as independent replicates through time and within a site ($P < 0.05$, data not shown). To meet criteria for normality, DOC from non-fumigated K_2SO_4 extracts, $NO_3^- - N$, $NH_4^+ - N$, and net mineralizable N were transformed by $\log_e$(variable + 1) and Total C was transformed by $x^{0.25}$. Data for these variables are reported as transformed values. MBC, gravimetric water content, and microbial respiration were not $\log_e$ transformed. Pairwise comparisons were performed using a protected LS Means and adjusted Bonferroni test for mean separation at the $P \leqslant 0.01$ significance level. The $P \leqslant 0.01$ significance level was chosen to control for type I errors due to multiple GLM analyses.

The relationship between microbial community composition, soil N and C pools and microbial activity was analyzed by Canonical Correspondence Analysis (CCA) (CANOCO, version 3.11.5, Microcomputer Power, Ithaca, NY). CCA permits direct analysis of PLFA profiles in relation to specific environmental variables (e.g., soil characteristics, site and management factors). It constrains ordination axes to be linear combinations of environmental variables, and will maximize the dispersion of the PLFA scores (ter Braak, 1987). Soil characteristics are represented by vectors. Vectors of greater magnitude and forming smaller angles with an ordination axis are more strongly correlated with that ordination axis. High scores of

Table 2
Means of live aboveground plant biomass

Live plant biomass ($g\,m^{-2}$)		Relict Grassland		Annual Grassland		Agricultural Grassland		Restored Grassland	
	n	x	±SE	x	±SE	x	±SE	x	±SE
Fall	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Winter	3	32.3	5.3	43.3	4.7	55.1	4.6	32.8	7.9
Spring	3	181.8	1.1	133.6	17.8	67.6	7.6	115.3	4.7

Table 3
Means of soil characteristics

		Relict Grassland[1]		Annual Grassland		Agricultural Grassland		Restored Grassland	
	n	x	±SE	x	±SE	x	±SE	x	±SE
Total C[£] (% g/g)	8	1.10^{a}	0.04	1.13^{a}	0.02	1.05^{a}	0.02	1.07^{a}	0.02
Total N (% g/g)	8	0.16^{a}	0.02	0.17^{a}	0.02	0.13^{a}	0.01	0.14^{a}	0.01
X–K ($cmol_c\,kg^{-1}$)	8	0.39^{a}	0.05	1.00^{b}	0.06	0.71^{c}	0.06	0.34^{a}	0.03
X-Ca ($cmol_c\,kg^{-1}$)	8	21.5^{a}	0.5	5.6^{b}	0.3	3.8^{c}	0.1	5.7^{b}	0.2
X-Mg[§]($cmol_c\,kg^{-1}$)	8	1.34^{a}	0.04	0.61^{bc}	0.02	0.70^{b}	0.03	0.58^{c}	0.02
CEC	8	34.7^{a}	1.0	16.9^{b}	0.4	14.2^{c}	0.4	16.5^{bc}	0.7
pH	8	6.9^{a}	0.1	6.0^{b}	0.1	5.5^{c}	0.1	6.1^{b}	0.1
% Sand (g/g)	3	68.3^{a}	2.2	70.0^{a}	0.0	80.3^{b}	0.9	79.7^{b}	0.3
% Silt (g/g)	3	17.3^{a}	0.9	21.3^{b}	0.3	14.0^{ac}	1.0	13.7^{c}	0.7
% Clay (g/g)	3	14.3^{a}	1.3	8.7^{b}	0.3	5.7^{c}	0.3	6.7^{b}	0.3

[1]Letters indicate significant difference between means using a one-way GLM for effect of site and pairwise mean comparisons with an adjusted Bonferroni test.
[§]Indicates that X-Mg has been transformed by $ln(x+1)$ and
[£]Indicates that Total C has been transformed by $x^{0.25}$ to meet conditions of normality for analysis by GLM.

absolute value for a given PLFA or a given site on a CCA axis indicate that it is highly related to the axis and to the environmental variable(s) exhibiting high correlation to the axis. All variables of soil N and C pools and microbial activity were tested for significant contribution to the explanation of the variation in the PLFA data with the Monte Carlo permutation test ($P \leqslant 0.05$) associated with the forward selection subroutine in CANOCO. Fifty-seven PLFA were used to describe the soil microbial community. PLFA were included in the analysis if they were clearly associated with a specific site or season by inspection or if they were present in all soil samples. This approach is similar to that used by plant ecologists when describing plant communities. Less common species have been shown to be important in distinguishing plant community types when using multivariate analysis (Kent and Coker, 1992).

3. Results

3.1. Plant community

Plant species richness was higher in the 'Restored Grassland' than the 'Relict Grassland', both for native and non-native species (Table 1). The 'Annual Grassland' had the lowest number of species, and only one native species. It was composed entirely of annual species, whereas the restored perennial grassland had two perennials (*N. pulchra* and *Elymus glaucus*). Three perennial species were identified at the 'Relict Grassland' (*N. pulchra*, *Dichelostemma capitatum*, and *Eriogonum* spp.).

Aboveground plant biomass increased from fall to spring (Table 2). In the spring, plant biomass was highest in 'Relict Grassland', followed by 'Annual Grassland', 'Restored Grassland', and 'Agricultural Grassland'.

3.2. Soil characteristics

Soil texture was generally similar between sites. 'Relict Grassland' and 'Annual Grassland' soils had similar sand content (Table 3). In 'Agricultural Grassland' and 'Restored Grassland', sand content was slightly higher than in 'Relict Grassland' and 'Annual Grassland'. The <2 mm fraction of 'Relict Grassland' had a sandy loam texture due to its greater clay content than the other sites, while 'Annual Grassland' had a sandy loam bordering on loamy sand texture. 'Restored Grassland' and 'Agricultural Grassland' were loamy sands. Total C and N did not differ among sites. Exchangeable K^+, Ca^{2+}, and Mg^{2+} differed among the four sites, with 'Relict Grassland' having higher values for all but exchangeable K^+. Soil pH also was higher in 'Relict Grassland' than in the other sites. CEC was ca. two-fold greater in 'Relict Grassland' than in

Table 4
Means of gravimetric soil water content ($g\,g^{-1}$)

		Relict Grassland		Annual Grassland		Agricultural Grassland		Restored Grassland	
	n	x	$\pm$SE	x	$\pm$SE	x	$\pm$SE	x	$\pm$SE
Plant[1]									
Fall	4	0.197[a]	0.018	0.121[a]	0.007	0.135[a]	0.004	0.124[a]	0.012
Winter	4	0.192[a]	0.015	0.134[a]	0.004	0.149[a]	0.006	0.127[a]	0.006
Spring	4	0.099[b]	0.009	0.099[b]	0.006	0.078[b]	0.006	0.069[b]	0.006
Removal									
Fall	4	0.155	0.006	0.116[a]	0.007	0.139	0.004	0.111[a]	0.008
Winter	4	0.171	0.012	0.126[a]	0.009	0.134	0.005	0.107[a]	0.007
Spring	4	0.150	0.017	0.089[b]	0.008	0.098	0.012	0.070[b]	0.009

[1]Superscript letters indicate significant differences between seasonal means within 'plant' or 'removal' plots for a given site (Three-way general linear model and pairwise mean comparisons with an adjusted Bonferroni test, $P \leqslant 0.01$).

the other three sites, which shared similar CEC values (Table 3).

3.3. Soil moisture

Soil moisture differed between land-use types and generally was greater in fall and winter and decreased in spring (Table 4). Soils at all sites in the spring were between 30% and 50% drier than those in fall and winter, irrespective of plant cover. Moisture was lower in 'plant' compared to 'removal' soils in both 'Relict Grassland' and 'Agricultural Grassland' in the spring and higher under plants than in 'removal' in 'Relict Grassland' in the fall (all comparisons not shown).

3.4. Soil carbon pools

'Relict Grassland' had the greatest overall MBC, followed by 'Annual Grassland', and then 'Agricultural Grassland' and 'Restored Grassland' (Fig. 1). Their respective mean values $\pm$ SE across all seasons and both zones were 440.7 ± 44.7, 283.0 ± 21.0, 155.7 ± 12.4, $137.0 \pm 11.8\,\mu g\,C\,g^{-1}$. The two grassland soils not disturbed by recent tillage, 'Relict Grassland' (never tilled) and 'Annual Grassland' (tilled in 1960s), had greater MBC than the tilled soils, 'Restored Grassland' (tilled 3 years prior to sampling) and 'Agricultural Grassland' (tilled 5 years prior to sampling).

In the 'Relict Grassland' site, MBC was significantly lower in adjacent interstitial areas ('removal': $324.0 \pm 27.2\,\mu g\,C\,g^{-1}$ vs. 'plant': $557.2 \pm 71.7\,\mu g\,C\,g^{-1}$, $n = 12$; Fig. 1). The other sites with bunchgrasses also had lower MBC in areas where plants had been removed. This was slightly greater for 'Restored Grassland' ('removal': $121.0 \pm 11.3\,\mu g\,C\,g^{-1}$ vs. 'plant': $190.4 \pm 17.2\,\mu g\,C\,g^{-1}$) than for 'Agricultural Grassland' ('removal': $123.2 \pm 15.0\,\mu g\,C\,g^{-1}$ vs. 'plant': $150.9 \pm 8.0\,\mu g\,C\,g^{-1}$). There was no effect of plant presence on MBC in 'Annual Grassland'.

When examining effects of season, MBC in 'Relict Grassland' decreased from fall to winter, and from winter to spring (Fig. 1). In 'Agricultural Grassland', MBC decreased between 30% and 50% from fall to winter. In both these sites, MBC was consistently higher in 'plant' than 'removal' soils in each season except spring, when MBC was similar between 'plant' and 'removal' soils. Consistent temporal changes in MBC did not occur in 'Restored Grassland' or 'Annual Grassland' (Table 6).

Higher DOC concentrations ($\log_e$ scale) under *N. pulchra* only occurred in 'Relict Grassland', the site that supported the oldest perennial bunchgrasses ('removal': $3.3 \pm 0.2\,\mu g\,C\,g^{-1}$, 'plant': $3.8 \pm 0.1\,\mu g\,C\,g^{-1}$, $n = 12$, Fig. 1). Site means of DOC including both zones were greatest in 'Relict Grassland' ($3.60 \pm 0.15\,\mu g\,C\,g^{-1}$) and 'Annual Grassland' ($4.10 \pm 0.12\,\mu g\,C\,g^{-1}$), followed by 'Restored Grassland' ($3.14 \pm 0.09\,\mu g\,C\,g^{-1}$) and 'Agricultural Grassland' ($2.87 \pm 0.10\,\mu g\,C\,g^{-1}$). 'Relict Grassland' and 'Restored Grassland' were the only sites to show decreases of 25–35% in DOC between fall and spring when 'plant' and 'removal' were pooled by season (data not shown). Neither plant presence nor season had a consistent effect on DOC across the sites (Tables 5 and 6). Thus, land-use history was the most important variable explaining concentrations of DOC.

The presence of *N. pulchra* also affected microbial respiration in 'Relict Grassland' as it was 55% greater under *N. pulchra* than in interstitial areas where annual plants had been removed (Fig. 1, Table 6). This trend was also true for 'Agricultural Grassland', in which microbial respiration was ca. 75% greater in soil under *N. pulchra* than in 'removal' soils when all seasonal data were pooled (data not shown). There were no detected differences in microbial respiration between 'plant' and 'removal' soils in 'Restored Grassland' and 'Annual Grassland'.

Microbial respiration in 'removal' soils of 'Relict Grassland' was strongly affected by season (Fig. 1, Table 6). It was almost twice as great in spring as in fall or winter, even though no temporal change in 'plant' soils occurred. Microbial respiration in 'plant' soils of 'Agricultural Grassland' nearly doubled between fall and winter, while this increase was delayed until spring in 'removal' soils. No

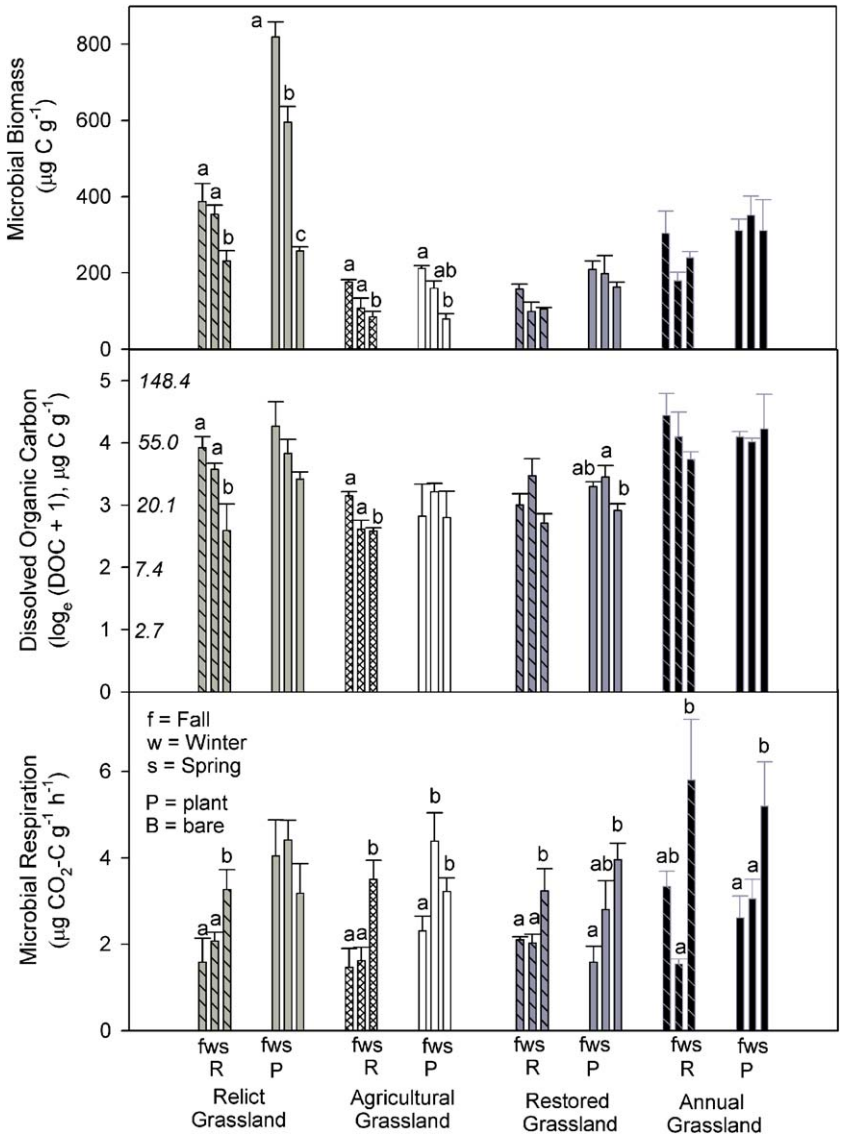

Fig. 1. Soil microbial biomass C (MBC), dissolved organic carbon (DOC) and microbial respiration for the four sites, 0–12 cm depth. "f", "w", "s" refer to seasons, "R" refers to 'removal' and "P" to 'plant' treatments. Letters indicate significant differences between seasonal means of a given plant or removal treatment for that site. Groups of bars without letters are not significantly different from each other. Italicized numbers on the *y*-axis of the DOC graph indicate backtransformed values for the transformed $\log_e$ data. Pairwise comparisons by LS Means and adjusted Bonferroni mean separation test ($P \leqslant 0.01$). See Table 4 for complete GLM results.

Table 5
Analysis of variance results

	Land use[a]	Season	±Plant	Land use *x* season	Land use *x* ±plant	Season *x* ±plant	Land use *x* season *x* ±plant
NO_3^--N ($\mu g\,g^{-1}$)	ns	***	ns	***	ns	†	ns
NH_4^+-N ($\mu g\,g^{-1}$)	**	***	ns	***	ns	ns	ns
Net mineralizable N (NH_4^+-N, $\mu g\,g^{-1}$)	***	*	**	***	*	ns	†
Microbial biomass C ($\mu g\,C\,g^{-1}$)	***	***	***	***	*	**	***
DOC ($\mu g\,C\,g^{-1}$)	**	**	†	ns	ns	ns	ns
Respiration ($\mu g\,CO_2$-C g^{-1})	*	***	**	**	†	*	ns
% Moisture ($g\,g^{-1}$)	***	**	ns	ns	ns	**	*

Asterisks indicate level of significance: $*P \leqslant 0.01$, $**P \leqslant 0.001$, $***P \leqslant 0.0001$. Main effects and interactions that were not significant are indicated by "ns". "†" indicates that main effects and interactions were significant at $P \leqslant 0.05$.

[a]Determined by GLM where $\alpha = 0.01$.

Table 6
Analysis of variance results by individual land use.

	NO_3^--N ($\mu g\,g^{-1}$)	NH_4^+-N ($\mu g\,g^{-1}$)	Net mineralizable N ($\mu g\,g^{-1}\,wk^{-1}$)	Microbial biomass C ($\mu g\,C\,g^{-1}$)	DOC ($\mu g\,C\,g^{-1}$)	Respiration ($\mu g\,CO_2$-$C\,g^{-1}\,h^{-1}$)	Soil moisture ($g\,g^{-1}$)
			Relict Grassland				
Season[a]	**	ns	*	***	†	*	*
±Plant	ns	†	*	***	†	*	ns
Season x ±plant	ns	ns	ns	**	ns	*	*
			Annual Grassland				
Season	ns	**	ns	ns	ns	*	**
±Plant	ns	ns	ns	ns	ns	ns	ns
Season x ± plant	ns	ns	ns	ns	ns	ns	ns
			Agricultural Grassland				
Season	*	ns	*	*	ns	*	**
±plant	ns	ns	***	*	ns	**	ns
Season x± plant	ns	ns	*	*	ns	†	*
			Restored Grassland				
Season	***	*	*	ns	*	**	**
±plant	ns	ns	ns	**	ns	ns	ns
season x ±plant	ns	ns	ns	ns	ns	ns	ns

Asterisks indicate level of significance: *$P \leqslant 0.01$, **$P \leqslant 0.001$, ***$P \leqslant 0.0001$. Interactions that were not significant are indicated by "ns". "†" indicates that main effects and interactions were significant at $P \leqslant 0.05$.

[a]Determined by GLM where $\alpha = 0.01$.

such difference occurred at the other sites. In both 'plant' and 'removal' soils of 'Restored Grassland' and 'Annual Grassland', microbial respiration nearly doubled between winter and spring.

3.5. Soil nitrogen pools

The presence of *N. pulchra* had no effect on soil $NO_3^- - N$ ($\log_e$ scale) pools in 'Relict Grassland' (Fig. 2, Table 6). This was also true in 'Agricultural Grassland', 'Restored Grassland', and for annual species in 'Annual Grassland'. In 'Relict Grassland', 'Restored Grassland' and 'Agricultural Grassland', soil $NO_3^- - N$ pools decreased from fall to spring, but this did not occur in 'Annual Grassland'.

The only site that showed an effect of plant presence on soil $NH_4^+ - N$ was 'Relict Grassland' ($\log_e$ scale, Fig. 2, Table 6). Here, $NH_4^+ - N$ under *N. pulchra* was ca. 30% greater than in 'removal' soil ($0.70 \pm 0.07\,\mu g\ NH_4^+ - N\,g^{-1}$ in 'removal' vs. $0.93 \pm 0.09\,\mu g\ NH_4^+ - N\,g^{-1}$ in 'plant'), but these values were relatively low. Not all sites acted in concert as seasons changed (Table 5). Pools of $NH_4^+ - N$ in 'Relict Grassland' and 'Agricultural Grassland' did not change with season (Fig. 2; Table 6). Seasonal changes in the other two sites were not consistent with each other. In 'Restored Grassland', soil $NH_4^+ - N$ decreased by half from fall to winter in 'plant' and 'removal' soils, but in 'removal' soils of 'Restored Grassland', soil $NH_4^+ - N$ doubled between winter and spring. In 'Annual Grassland', $NH_4^+ - N$ pools in 'plant' and 'removal' soils increased two to five times from fall to spring.

In 'Relict Grassland', net mineralizable N under *N. pulchra* was ca. 80% greater than in interstitial areas (Fig. 2, Table 6). In 'Agricultural Grassland', net mineralizable N was ca. 30% greater under *N. pulchra* than in 'removal' soils. There was no effect of plant presence on net mineralizable N in 'Restored Grassland' and 'Annual Grassland'. 'Relict Grassland' was the only site to show seasonal decreases in net mineralizable N (ca. 50%) in 'removal' soils, and this occurred between winter and spring. In all sites except 'Relict Grassland', net mineralizable N tended to increase between 30% and 50% over the growing season in soils supporting plants but not in interstitial bare areas.

3.6. Microbial community composition

Microbial communities clustered according to land use when PLFA from all sites and seasons were analyzed together by CCA (Fig. 3). The variation in PLFA profiles explained by the first two axes was 34.2%. 'Relict Grassland', 'Annual Grassland', 'Restored Grassland' and 'Agricultural Grassland' clustered separately from right to left, respectively, along the first axis, with 'Annual Grassland' shifted in the negative direction along the second axis. Vectors for GWC, total PLFA and MBC ($P \leqslant 0.05$, Monte Carlo) were associated with the first axis and pointed in the positive direction. Thus, higher values for these variables were more positively related to the undisturbed grassland sites. Higher net mineralizable N, DOC, $NH_4^+ - N$, microbial respiration (Monte Carlo, $P \leqslant 0.05$) and nitrate ($P = 0.38$) were associated with the second axis and microbial communities from 'Annual Grassland'.

When each individual site was analyzed separately to determine the effect of plant presence and season, microbial communities clustered first by season and then,

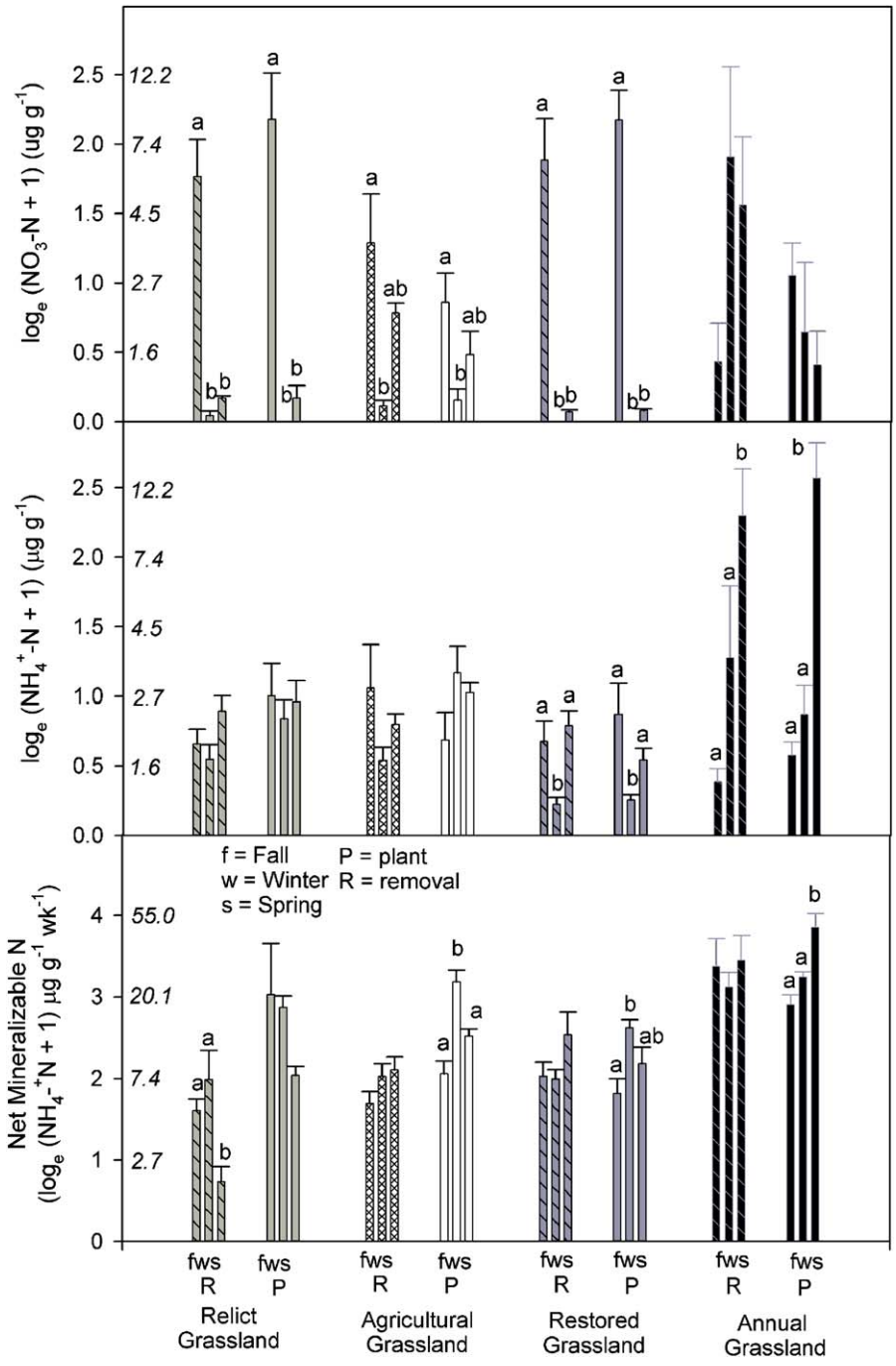

Fig. 2. Soil ammonium, nitrate and net mineralizable N for the four sites, 0–12 cm depth. Letters indicate significant differences between seasonal means of a given plant or removal treatment for that site. Groups of bars without letters are not significantly different from each other. Italicized numbers on the *y*-axis indicate backtransformed values for the transformed log$_e$ data. Pairwise comparisons by LS Means and adjusted Bonferroni mean separation test ($P \leqslant 0.01$). See Table 4 for complete GLM results.

in some cases, by 'plant' and 'removal' treatments (Figs. 4 and 5). In the CCA of 'Relict Grassland', 41.7% of the variation was explained by the first two axes. In the three remaining sites, variation explained by the first two axes ranged between 23.2% and 29.4%. The presence of *N. pulchra* influenced microbial communities in 'Relict Grassland' in the spring and in 'Agricultural Grassland' in the winter and spring, as indicated by the separate clusters of microbial communities in 'plant' vs. 'removal' soils in these seasons (Fig. 4). The 'Annual Grassland' microbial communities in 'plant' soils were different from those in 'removal' soils in the spring (Fig. 5). In 'Agricultural Grassland', microbial communities in soils with plants in winter and spring tended to segregate on the negative side of the first axis, away from 'removal' soils.

Soil characteristics that were significant in explaining the variation in PLFA were correlated with the first axis and were associated with seasonal changes in microbial

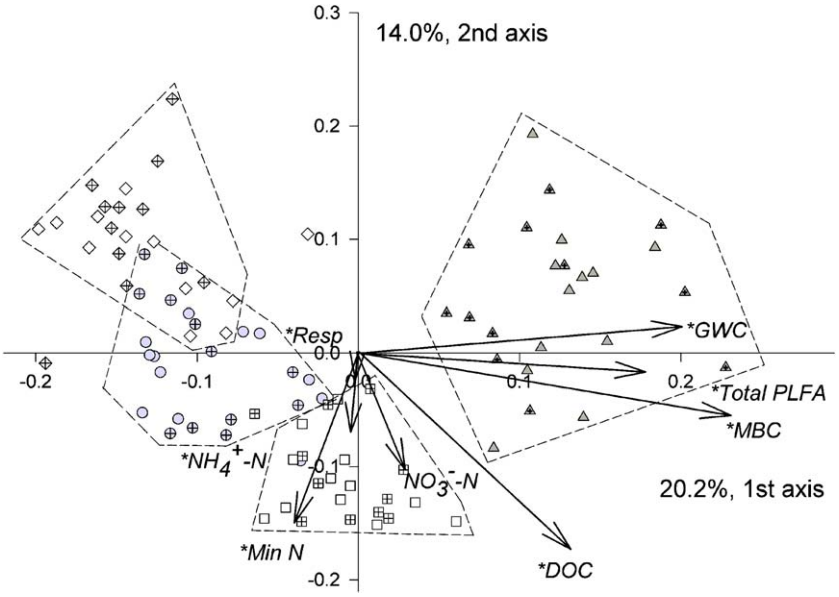

Fig. 3. CCA of microbial community composition at 0–12 cm depth across land-use types. Diamonds (◇) indicate 'Agricultural Grassland', circles (○) indicate 'Restored Grassland', squares (□) indicate 'Annual Grassland', and triangles (△) indicate 'Relict Grassland'. Symbols with "+" in the center are from 'plant' soils while open symbols are from 'removal' soils. Asterisks indicate that the environmental variable is significant ($P \leqslant 0.05$, Monte Carlo). Dashed polygons outline clusters of microbial communities by land-use type.

communities (Figs. 4 and 5). Total PLFA was the only significant environmental variable (Monte Carlo, $P \leqslant 0.05$) held in common among sites. In 'Relict Grassland', total PLFA tended to be positively associated with microbial communities under *N. pulchra* from fall and winter, but in the three other sites, it was positively associated with microbial communities in spring and winter. MBC and $NO_3^- - N$ were also significant in explaining variation in microbial communities in 'Relict Grassland'. Net mineralizable N and $NO_3^- - N$ in 'Restored Grassland', $NH_4^+ - N$ and MBC in 'Agricultural Grassland', and microbial respiration (i.e., 'Resp' in Figs. 4 and 5) in 'Annual Grassland' were significant in explaining variation in PLFA. Typically, higher soil $NO_3^- - N$ was positively associated with microbial communities from fall, while higher microbial respiration, $NH_4^+ - N$, Min N, and MBC were more often positively associated with microbial communities in winter and spring. However, little variation in the distribution of soil microbial communities in the respective CCAs was explained by microbial activity and soil C and N pools.

Of the 57 PLFA included in the CCA analyses of each individual site, 30 PLFA were identified among all four sites to be important in separating microbial communities by the presence and absence of vegetation and by season (data not shown). This was based on their CCA loading scores for individual PLFA (i.e., >2.00 and <-2.00) and their positive associations with microbial communities as determined from species biplots of individual PLFA (Myers et al., 2001). Of this group of 30 PLFA, 77% in 'Relict Grassland', 66% in 'Restored Grassland', and 57% in 'Annual Grassland' and 'Agricultural Grassland' were identified as important in separating microbial communities by plant presence and/or season. In other words, not all PLFA associated with 'plant' soils were the same across all sites. Typically, PLFA that were uniquely associated with either 'plant' or 'removal' soils occurred during the spring except in 'Restored Grassland', where no differentiation occurred between 'plant' or 'removal' soils.

Specific PLFA were important in distinguishing microbial community composition in 'plant' or 'removal' soils in the spring in three out of four sites, but these distinctive PLFA were not the same among sites. In 'Relict Grassland', markers for general bacteria (i.e., 12:0 2OH, 16:0 *n*OH, 12:0 3OH) and 17:1 ω5c were present in higher abundances under *N. pulchra* in spring. Soils under *N. pulchra* in 'Relict Grassland' in spring were also enriched in unknown marker sum2 (an unresolved mixture of 15:1 iso H, 13:0 3OH, and 15:1 iso I), 15:1ω8c, and 12:0. In 'removal' soil in 'Relict Grassland', a marker for *Thiobacillus* (i.e., iso17:1 ω5c; Dowling et al., 1986) and 16:1 ω11c contributed to differences in the spring.

In 'Agricultural Grassland', 'plant' soils in winter were different from 'removal' soils due to the higher abundance of markers for general bacteria (16:0 2OH, 10:0 2OH, 12:0 3OH), gram-positive bacteria (13:0 iso; Harwood and Russell, 1984), 17:1 ω5c and 15:1 ω8c. In spring, *N. pulchra* soil in 'Agricultural Grassland' had higher abundances of markers for gram-positive bacteria (anteiso 19:0 and iso 15:1; Harwood and Russell, 1984; Myers et al., 2001) and general bacteria (i.e., 16:1 2OH; Harwood and Russell, 1984). In 'removal' soil in 'Agricultural Grassland', iso 15:1 at 5, sum8 (an unresolved mixture of unknown 18.756 and

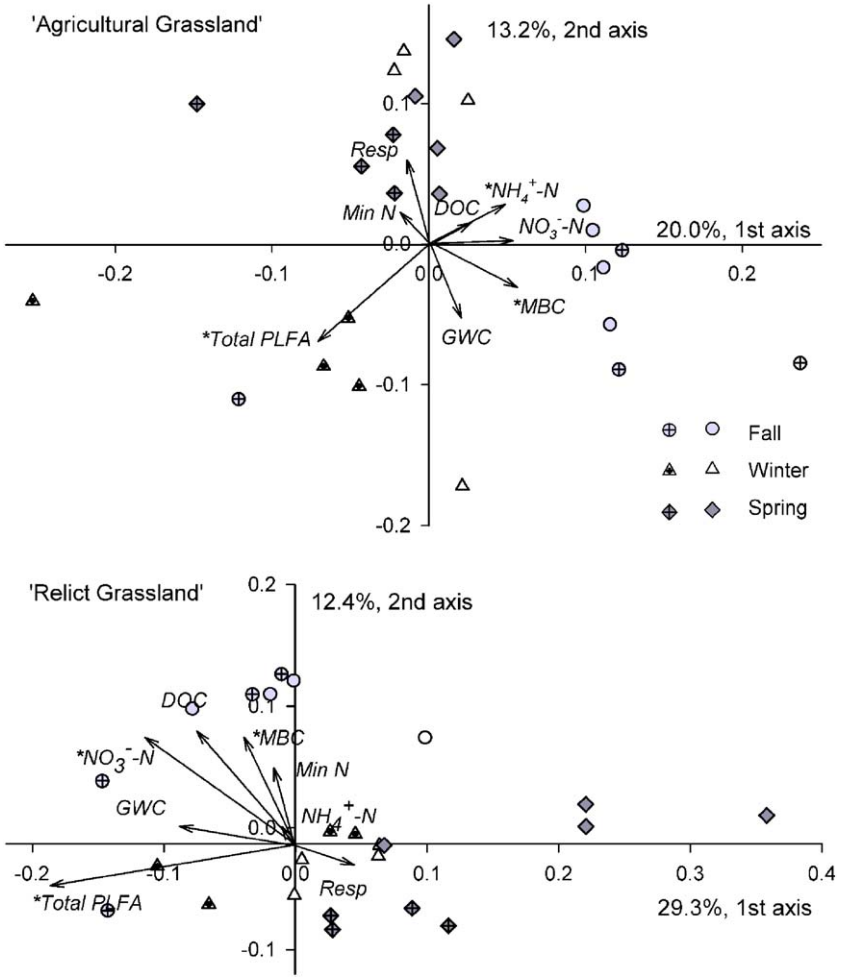

Fig. 4. CCAs of microbial community composition in the 'Agricultural Grassland' and 'Relict Grassland' at 0–12 depth. Asterisks indicate that the environmental variable is significant ($P \leq 0.05$, Monte Carlo). Symbols with "+" in the center are from 'plant' soils while open symbols are from 'removal' soils. Circles (○) indicate fall and triangles (△) indicate winter, diamonds (◇) indicate spring.

10:1 ω11c) and 15:1 ω8c contributed to differences in microbial communities in winter and spring.

'Restored Grassland', in contrast to 'Agricultural Grassland' and 'Relict Grassland', shared many PLFA between 'plant' and 'removal' soils in each season and showed little seasonal differentiation among microbial communities. Nonetheless, PLFA markers for general microeukaryotes and protozoa (i.e., 20:4 ω6,9,12,15; Zelles, 1997; Myers et al., 2001) and bacteria (i.e., 16:0 2OH, 14:0 2OH, 18:0 2OH; Harwood and Russell, 1984) were associated with 'plant' soils in fall in 'Restored Grassland', as were 18:0 iso and iso 15:1 at 5.

In 'Annual Grassland', PLFA markers were present for gram-negative bacteria (i.e., 16:1 ω5c; Nordby et al., 1981) and *Thiobacillus* (i.e., iso17:1 ω5c; Macalady et al., 2000) in 'plant' soils in fall. 16:1 ω5c also has been linked to mycorrhizae and type I methanotrophs (Nichols et al., 1985; Peacock et al., 2001). In spring, general bacterial markers like 16:0 2OH, 16:0 *n*OH and unknown marker 17:1 ω5c only were present under annual grasses in 'Annual Grassland'.

In summary, as seasons changed and plant growth increased, dominance of specific microbial markers across seasons within a given site shifted. Furthermore, microbial markers that distinguished samples from 'plant' and 'removal' plots differed across sites despite the presence of the same perennial bunchgrass species. Not only were there no characteristic similarities in markers due to *N. pulchra* across sites, many of the distinguishing patterns could not be attributed to a specific microbial group. Primarily, observed differences were in bacteria rather than fungal markers.

4. Discussion

In the relict perennial grassland, soils with the perennial bunchgrass *N. pulchra* had large and generally consistent responses of MBC, microbial respiration, soil $NH_4^+ - N$, DOC, net mineralizable N and soil moisture, compared to interstitial areas where annual plants had been removed. No other sites showed such marked effects between the two zones. Although some differences in microbial community

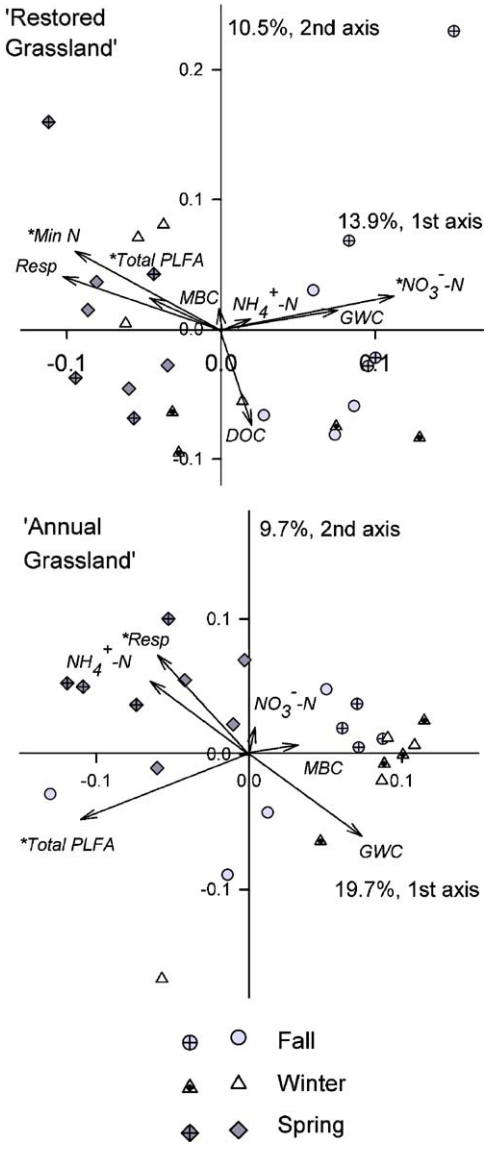

Fig. 5. CCAs of microbial community composition in 'Annual Grassland' and 'Restored Grassland' at 0–12 cm depth. Asterisks indicate that the environmental variable is significant ($P \leqslant 0.05$, Monte Carlo). See Fig. 4 caption for description of symbols.

composition occurred between the bunchgrass and bare interstitial zones, they were not pronounced, even in the relict perennial grassland, nor were they similar between sites with differing land-use history. This suggests that microbial communities are not strictly controlled by the presence of a given plant species, but rather are a result of many seasonal and abiotic factors, as well as the presence of roots and litter of minor annual species (Callaway et al., 2004). Clearly, historical land use creates a unique environment for microorganisms, alters soil biota, and changes biogeochemical processes, suggesting that restoration of plant community composition does not simply result in restoration of the microbial community of the relict ecosystem and its above- and belowground linkages (De Deyn et al., 2003; Steenwerth et al., 2003).

4.1. Influence of plants and season on soil resources and microbial activity

The responses of MBC and microbial respiration suggest that both biotic and abiotic factors were important in explaining the differences between sites. MBC under *N. pulchra* in the relict perennial grassland and perennial grass agriculture decreased with diminished soil moisture despite increases in plant growth. Microbial respiration was greater under *N. pulchra* in these two sites in fall and winter, a period when bunchgrasses broke dormancy and initiated new growth, suggesting that recent rhizodeposition affected microbial respiration. Abiotic factors may have exerted additional control over seasonal MBC levels and microbial respiration rates. Wet-dry cycles, which are a natural component of Mediterranean grasslands and can be accompanied by increases in available soil C, in conjunction with warmer ambient temperatures in spring, may partly explain why soil respiration increased in both 'plant' and 'removal' soils in this study even as soil moisture and microbial biomass decreased (Van Gestel et al., 1993a b; Appel, 1998; Burger et al., 2005).

Few differences in DOC were observed. The only site that exhibited a weak effect of plant presence was the never-tilled, relict perennial grassland, the site that supported the longest-lived bunchgrasses. High root length density (300 m kg^{-1} in annual grassland and 250 m kg^{-1} in the restored perennial grassland at 0–15 cm a year after this study (Potthoff et al., 2005)) would be expected to affect DOC pools in soils with plants, especially because live roots were not found in 'removal' soils. However, even short-term tillage practices such as those used in the restoration of the native perennial grassland can cause significant reductions in labile soil C pools (Su et al., 2004, Potthoff et al., 2005), and cultivation history can have a lasting imprint on soil C recovery (Woods, 1989; Puget and Lal, 2005). Furthermore, recently disturbed sandy soils, such as those in this study, possess few colloidal surfaces from clays that help stabilize soil organic matter (Burke et al., 1989; Saggar et al., 1996). Effects of plant presence on DOC in the relict perennial grassland are likely due to relatively long-term deposition of organic matter compared to the other perennial sites as well as absence of previous cultivation.

Higher concentrations of $NH_4^+ - N$ under perennial bunchgrasses were accompanied by higher net mineralizable N. Due to low $NH_4^+ - N$ concentrations and a lack of a 'plant' effect in the annual grassland and restored perennial grassland, it is likely that the $NH_4^+ - N$ was immobilized and turned over rapidly by microbes in both 'removal' and 'plant' soils (Burger and Jackson, 2003).

The effect of *N. pulchra* on soil resource pools and microbial activity between 'plant' and 'removal' soils tended to be strongest in the relict perennial grassland, even though increases in MBC under bunchgrasses were present 3 years after establishment of perennial bunchgrasses in the restored perennial grassland and after 5 years

in perennial grass agriculture. However, bunchgrasses at the perennial grass agriculture site and the relict grassland were similar in size despite differences in age. Due to intermittent fertigation (i.e., fertilizer applied via irrigation lines) and irrigation, perennial grass agriculture supported relatively rapid growth rates of grasses, as indicated by the more extensive root system, higher standing dead aboveground biomass and larger basal diameter of perennial bunchgrasses compared to the restored perennial grassland, despite similarity in age (Potthoff et al., 2005; personal observation). Although bunchgrasses in the relict perennial grassland and perennial grass agriculture site were similar in size, bunchgrasses at the relict perennial grassland still had greater effects on soil resource pools. *N. pulchra* in the rare, relict bunchgrass stands in the region has been documented to be >75 years old, suggesting that plant associations with soil biota have resulted from long-term interactions (Hamilton et al., 2002).

In 'Annual Grassland', a site composed of ruderal annual plant species, soil resource pools did not behave similarly to the sites with perennial bunchgrasses. Greater soil resources and microbial activity occurred under annual grasses compared to the restored perennial grassland and perennial grass agriculture. Soil C and N pools were more similar among 'plant' and 'removal' soils in annual grassland than in the relict perennial grassland and perennial grass agriculture, demonstrating that decreases in spatial heterogeneity of soil resources can occur with conversion of perennial grasslands to old-field annual grasslands. Differences in soil resources and microbial activity between old-field annual and perennial grassland systems may be attributed to species phenology, life strategy (r-selected vs. k-selected), and tissue quality of annual grasses and forbs compared to *N. pulchra* (Hooper and Vitousek, 1998; Corbin and D'Antonio, 2004a).

4.2. Response of soil microbial community composition to land-use history and plant presence

Although plants can influence soil microbial communities (Kowalchuk et al., 2002; Callaway et al., 2004; Batten et al., 2006), these responses are not necessarily consistent across the gradient in land-use intensification. Primarily, land-use history appears to limit the range in which the microbial community composition oscillates over the three seasons, despite the presence or absence of vegetation (Fig. 3). The CCA of all sites indicated that land-use history was the dominant factor distinguishing microbial communities, which is consistent with previous studies (Buckley and Schmidt, 2001; Steenwerth et al., 2003). A clear disturbance gradient reflecting time since tillage and management inputs was distinguishable; microbial communities of the perennial grass agriculture site, restored perennial grassland, annual grassland, and the relict perennial grassland were consecutively ordered along the first axis of the CCA biplot. This disturbance gradient was associated with differences in nutrient pools such that the annual grassland had higher N availability or turnover and retention than any of the perennial-dominated sites, and C availability was highest in the relict grassland and decreased with more recent soil disturbance. The decrease in soil C with more recent disturbance and increasing cultivation intensity exhibited in this study has been observed repeatedly (Lal, 2002), while invasion of perennial grasslands by annual plant species has been associated with shifts in soil N dynamics and the composition of nitrifiers, a component of the soil microbial community (Booth et al., 2003; Corbin and D'Antonio, 2004b; Hawkes et al., 2005).

Despite the presence of the same perennial bunchgrass species, different PLFA played a role in distinguishing the microbial community composition between seasons and under *N. pulchra* (e.g., relict perennial grassland and perennial grass agriculture) or annual grasses in each site of distinct land-use history. The occurrence of shifts in individual PLFAs contributing to seasonal differences is consistent with seasonal studies of soil microbial communities in temperate forest systems, upland grasslands and organic and conventional agriculture (Bossio et al., 1998; Bardgett et al., 1999; Myers et al., 2001). Furthermore, dissimilarity between microbial communities in rhizosphere soils from plants of the same species has been observed previously (Westover et al., 1997). Through feedbacks between the microorganisms associated with a given plant and the resultant rhizodeposition, soil microbial community composition can differentiate on a plant to plant basis (Westover et al., 1997), as suggested by the high variability in the microbial communities associated with different individual plants of the restored perennial grassland (Fig. 4). Microclimate, transpiration rates and plant growth rates undoubtedly play a role in creating different above- and belowground relationships (Wardle et al., 2004). Coupled with the tremendous diversity in soil microbial communities and the effect of land-use history on the soil microbial community and nutrients, these factors may partly explain why the same plant species, *N. pulchra*, can differentially affect soil resource pools and microbial communities across sites. In this case, even though *N. pulchra* can influence soil resource pools and PLFA, effects from land-use conversion appear to more strongly affect microbial community composition as well as soil C and N dynamics.

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